Cardiovascular responses to microinjections of urocortin 3 into the nucleus tractus solitarius of the rat

Takeshi Nakamura, Kazumi Kawabe, and Hreday N. Sapru

Department of Neurological Surgery, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey

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Nakamura T, Kawabe K, Sapru HN. Cardiovascular responses to microinjections of urocortin 3 into the nucleus tractus solitarius of the rat. Am J Physiol Heart Circ Physiol 296: H325–H332, 2009. First published December 5, 2008; doi:10.1152/ajpheart.01044.2008.—Urocortin 3 (Ucn3) is a new member of the corticotropin-releasing factor (CRF) peptide family and is considered to be a specific and endogenous ligand for CRF type 2 receptors (CRF2Rs). The presence of CRF2Rs has been reported in the nucleus tractus solitarius (NTS) of the rat. It was hypothesized that the activation of CRF2Rs in the medial NTS (mNTS) may play a role in cardiovascular regulation. This hypothesis was tested in urethane-anesthetized, artificially ventilated, adult male Wistar rats. Microinjections (100 nl) of Ucn3 (0.03, 0.06, 0.12, and 0.25 mM) into the mNTS of anesthetized rats elicited decreases in mean arterial pressure (MAP: 5.0 ± 1.0, 21.6 ± 2.6, 20.0 ± 2.8, and 12.7 ± 3.4 mmHg, respectively) and heart rate (HR: 7.8 ± 2.6, 46.2 ± 9.3, 34.5 ± 8.4, and 16.6 ± 4.9 beats/min, respectively). Microinjections of artificial cerebrospinal fluid (100 nl) into the mNTS did not elicit cardiovascular responses. Maximum decreases in MAP and HR were elicited by 0.06 mM concentration of Ucn3. Cardiovascular responses to Ucn3 were similar in unanesthetized midcollicular decerebrate rats. A bilateral vagotomy completely abolished Ucn3-induced bradycardia. The decreases in MAP and HR elicited by Ucn3 (0.06 mM) were completely blocked by astressin (1 mM; nonselective CRFR antagonist) and K41498 (5 mM; selective CRF2 antagonist). Microinjections of Ucn3 (0.06 mM) into the mNTS decreased the efferent greater splanchnic nerve activity. After the blockade of CRF2Rs in the mNTS, a Ucn3-induced decrease in the efferent sympathetic nerve discharge was abolished. These results indicate that Ucn3 microinjections into the mNTS exerted excitatory effects on the mNTS neurons via CRF2Rs, leading to depressor and bradycardic responses.

blood pressure; corticotropin-releasing factor receptor antagonists; heart rate; sympathetic nerve activity; urocortin; nucleus tractus solitarius

THE CORTICOTROPIN-RELEASING factor (CRF; also known as corticotropin-releasing hormone) is the main mediator of stress responses. Recently, three other peptides have been included in the CRF peptide family. These peptides include urocortin 1 (Ucn1), urocortin 2 (Ucn2), and urocortin 3 (Ucn3). Ucn1, formerly known as Ucn (10, 43), is present in several brain areas including the hypothalamus (16, 20, 27, 45). Intracerebroventricular injections of Ucn1 decrease water and food intake (37), increase anxiogenic behavior (26), and inhibit edema due to thermal injury (40). Ucn2 is also present in the hypothalamus (32); it is sometimes referred to as “stresscopin-related peptide,” which is Ucn2 with a 5-amino acid extension at the NH2-terminal. It has been implicated in the central regulation of appetite and autonomic functions. Ucn3 and stresscopin were discovered independently by two research groups (15, 22) while searching the public human genome databases. The two groups interpreted the posttranslational processing sites differently and arrived at a different amino acid composition of these peptides (15, 22). Ucn3 is sometimes referred to as “stresscopin,” which is Ucn3 with 2-amino acids extended from the NH2-terminal (23, 24). Ucn3-expressing neurons are present predominantly in the medial amygdala, the hypothalamic median preoptic nucleus, and the rostral perifornical area lateral to the hypothalamic paraventricular nucleus (23). Ucn3 fibers are present mainly in the hypothalamus (medial preoptic, paraventricular, and ventromedial nuclei) and limbic structures (lateral septum, posterior region of bed nucleus of stria terminalis, and medial nucleus of amygdala) (23). Two major subtypes of CRF receptors (CRFRs), CRF receptor types 1 (CRF1-R) and 2 (CRF2-R), have been identified (8, 11, 14, 31). These plasma membrane receptors are seven-transmembrane domain G protein-coupled receptors and stimulate adenylate cyclase activity. In the rat, CRF2-Rs are distributed widely in the brain with high densities in the cerebral cortex, hippocampus, amygdala, cerebellum, olfactory bulb, and sensory relay nuclei (5, 8, 42). CRF1-Rs are distributed in the paraventricular nucleus of the hypothalamus, lateral septum, amygdala, hippocampus, olfactory bulb, and nucleus tractus solitarius (NTS) (5, 8, 42). Ucn1 mediates its actions via both CRF1-Rs and CRF2-Rs, whereas Ucn2 and Ucn3 mediate their actions via CRF2-Rs (14).

Information regarding the cardiovascular effects of Ucn3 is limited. According to one report, intracerebroventricular injections of stresscopin, which has a close structural similarity to Ucn3, elicited an increase in blood pressure (BP) and heart rate (HR) (7). Since intracerebroventricular injections reach several brain areas, it is difficult to dissect out the site of cardiovascular actions in specific cardiovascular regulatory brain regions using this route of administration. Therefore, direct microinjections of substances into specific brain regions have been used to elucidate their sites of cardiovascular action. With the use of this technique, microinjections of Ucn1 and Ucn2 into the medial NTS (mNTS) have been reported to elicit depressor and bradycardic responses (46). There is only one report in which Ucn3-immunoreactive neurons and their projections have been identified (23). According to this report, Ucn1, but not Ucn3, fibers are present in the NTS (23). However, the presence of CRF2Rs in the NTS...
rats (Charles River, Wilmington, MA) weighing 300–360 g (electronic).
The tidal volume and frequency were adjusted on the
by the BP waves. Mean arterial pressure (MAP) was derived elec-
monitoring BP. HR was monitored by a tachograph that was triggered
response to pinching of a hind paw indicated that the rats were
(34) and CRF₂Rs in the NTS and area postrema (21, 42) has
been reported. Since Ucn3 is a highly specific agonist for
CRF₂Rs, it has been suggested that this neuropeptide may be
the endogenous ligand for these receptors (15, 22–24). Ucn3
has been implicated as an endogenous anxiolytic brain neu-
ropetide that attenuates behavioral response to stress (17, 44).
Understanding the nature and sites of cardiovascular actions of
Ucn3 is important, considering that systemic BP is known to be
be elevated by persistent stress. In the present article, the cardio-
vascular actions of microinjections of Ucn3 into the mNTS were
investigated with the eventual aim of unraveling the physiological role of CRF₂Rs, if any, in this nucleus. This
information is likely to be important, considering that the
mNTS is the site where baroreceptor, chemoreceptor, and
cardiopulmonary afferents make their first synapse and that it
is known to play an important role in the regulation of cardio-
vascular function (35).

MATERIALS AND METHODS

General procedures. Experiments were done in adult male Wistar rats (Charles River, Wilmington, MA) weighing 300–360 g (n = 52). All animals were housed under controlled conditions with a 12-h:12-h light-dark cycle. Food and water were available to the animals ad libitum. The experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996) and with the approval of the Institutional Animal Care and Use Committee of this university.

The general procedures have been described in detail elsewhere (28). Briefly, the rats were anesthetized with an inhalation of isofluran (2 to 5% in 100% oxygen), the trachea was cannulated with polyethylene tubing (PE-240), and the rats were artificially ventilated using a rodent ventilator (model 683, Harvard Instruments, Holliston, MA). One of the femoral veins was cannulated, and urethane (1.2–1.4 g/kg) was injected intravenously in 12–15 aliquots at 2-min intervals (total volume of the anesthetic solution was 0.55–0.75 ml, injected over a period of about 25–30 min). Isofuran inhalation was terminated after the administration of 5 to 6 aliquots of urethane. The absence of a pressor response and/or the withdrawal of the limb in response to pinching of a hind paw indicated that the rats were properly anesthetized. One of the femoral arteries was cannulated for monitoring BP. HR was monitored by a tachograph that was triggered by the BP waves. Mean arterial pressure (MAP) was derived electronically. The tidal volume and frequency were adjusted on the ventilator to maintain the end tidal CO₂ at 3.5–4.5%. Rectal temperature was maintained at 37.0 ± 0.5°C using a temperature controller (model TCAT-2AC, Physitemp Instruments, Clifton, NJ). All the tracings were recorded on a polygraph (model 7D, Grass Instruments, West Warwick, RI).

Decerebration. This procedure has been described elsewhere (28). Anesthesia was induced and maintained by tracheal administration of isofuran as described earlier. The external and internal carotid and pterygopalatine arteries were ligated bilaterally to minimize bleeding. The rats were placed in a prone position in a stereotaxic instru-
ment (David Kopf Instruments, Tujunga, CA), the parietal bones were removed, the dura was incised, and the brain was transected at the midcollicular level. The portion of the brain rostral to the transection was removed by suction and the cranial cavity was loosely packed with cotton balls. The administration of isofuran was terminated at this time, and a stabilization period of 50–60 min was allowed after the decerebration.

Vagotomy. Vagotomy was necessary in experiments designed to investigate the role of parasympathetic innervation to the heart in mediating the bradycardic responses elicited by microinjections of Ucn3 into the mNTS. In these experiments, silk sutures were placed loosely around the vagus nerves bilaterally for subsequent identification and sectioning of the nerves.

Microinjections. The details of this technique are described else-
where (28). Briefly, the rats were placed in a prone position in a
stereotaxic instrument with bite bar 18 mm below the interaural line.
The microinjections were made using a dorsal approach. Four-bar-
relled glass micropipettes (tip size, 20–40 μm) were mounted on a
micromanipulator (model 1460 with an AP slide 1262, David Kopf
Instruments), and each barrel was connected via PE tubing to one of the channels on a picospritzer (General Valve Corp, Fairfield, NJ). The barrels contained l-glutamate (l-Glu), artificial cerebrospinal fluid (aCSF), Ucn3, and CRFR antagonist. The coordinates for the mNTS were 0.5–0.6 mm rostral and 0.5–0.6 lateral to the calamus scriptorius and 0.5–0.6 mm deep from the dorsal medullary surface. The sites eliciting depressor and bradycardic responses were identified by microinjections of l-Glu (5 mM). The volumes of all microinjections into the mNTS were 100 nl; the selection of these volumes was based on our previous studies in which these volumes of l-Glu elicited minimal responses from the mNTS (18). The volumes were pressure ejected (30–35 psi) and visually confirmed by the displacement of fluid meniscus in the barrel containing the solution using a modified binocular horizontal microscope with a graduated reticule in one eyepiece (model PZMH, World Precision Instruments, Sarasota, FL). The duration of microinjection was 5–10 s. Microinjections (100 nl) of aCSF (pH 7.4) into the mNTS were used as controls.

In the microinjection studies, a concentration response was first done in one group of rats to ascertain a maximally effective concentra-
tion of Ucn3. In a separate group of rats, this concentration of Ucn3 was then microinjected into the same mNTS site at least four times to test whether repeated microinjections exhibited tachyphylaxis. Actual data for the concentration response and tachyphylaxis experiments are presented in results.

Greater splanchnic nerve recording. The greater splanchnic nerve (GSN) was exposed using a retroperitoneal approach. A 1.5-cm long incision was made on left side, just caudal to the last rib, parallel to the vertebral column and about 1.5 cm lateral to the midline. The segment of the GSN immediately proximal to the celiac ganglion was identi-

fied using an operating microscope, sectioned at its junction with the celiac ganglion, and a few millimeters of the central end of the nerve were desheathed (28). The exposed portion of the nerve was placed on a bipolar electrode made of silver wire. The nerve, along with the tips of the electrode, was embedded in a silicone elastomer (Kwik-Sil, WPI), which was allowed to set for 5–10 min. The electrode was connected to a probe head stage (model Super-Z, CWE, Philadelphia, PA), and the whole nerve discharge was amplified (×20,000–30,000, using model BMA-830 amplifier, CWE) and filtered (100–5,000 Hz). The amplified signals were digitized (22 kHz) using Neuro-Corder (Cygnus Technologies, Delaware Water Gap, PA), visualized on an oscilloscope (model R5103N, Tektronix, Beaverton, OR), and stored together with BP and HR on a video cassette recorder. The whole nerve activity was full-wave rectified, and an integrated signal was obtained (time constant, 100 ms; model MA-821 Integrator, CWE). At the end of the experiment, the GSN was sectioned centrally, and the remaining activity was considered to be the noise level.

Histology. Typical sites of microinjections in the mNTS were
marked by microinjections of diluted India ink. The animals were perfused and fixed with 4% paraformaldehyde, and serial sections of the medulla were cut (40 μm) in a vibratome, mounted on slides, dehydrated, cleared, and stained with cresyl violet. The microinjection sites were identified using a microscope, and the sections were photographed and compared with a standard atlas (30).

Drugs and chemicals. The following drugs and chemicals were used: astressin (nonselective CRFR antagonist) (13, 21), K41498 (selective antagonist for CRF₂R) (21), Ucn3, l-Glu monosodium, l-phenylephrine hydrochloride, isofuran, and urethane. All of the solutions for the microinjections were freshly prepared in aCSF. The

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concentrations of different constituents in the aCSF were as follows: (in mM) 152 NaCl, 3 KCl, 1.2 CaCl₂, 0.8 MgCl₂, 3.4 dextrose, and 5 HEPES (298 ± 2 mosmol/kg). Where applicable, the concentration of drugs refers to their salts. The sources of different drugs and chemicals were as follows: astressin and Ucn3 (American Peptide, Sunnyvale, CA), K41498 (Tocris Cookson, Ellisville, MO), and isoflurane (Baxter Pharmaceutical Products, Deerfield, IL). All other drugs and chemicals were obtained from Sigma Chemicals (St. Louis, MO).

Statistical analyses. For comparison of MAP and HR responses, the mean and mean ± SE were calculated for maximum changes in these values in response to microinjections of Ucn3 or L-Glu into the mNTS. In the concentration-response studies, comparisons of the maximum decreases in MAP and HR in different groups of rats were made by using a one-way ANOVA followed by Tukey-Kramer’s multiple comparison. In experiments testing for tachyphylaxis and the effect of CRFR antagonists, the comparisons of the maximum decreases in MAP and HR elicited by microinjection of Ucn3 into the mNTS were made by using repeated-measures ANOVA followed by Tukey-Kramer’s multiple comparison test. Student’s paired t-test was used for comparisons of maximum decreases in MAP and HR elicited by the following: 1) microinjection of Ucn3 into the mNTS before and after the bilateral vagotomy and 1 microinjection of L-Glu into the mNTS before and after the microinjections of astressin or K41498. An unpaired t-test was used for comparisons of the maximum decreases in MAP and HR elicited by microinjection of Ucn3 into the mNTS in anesthetized and decerebrate rats. For the analysis of nerve activity, the control value represented the average amplitude of integrated GSN activity (GSNA) during a 35-s period before the microinjections of Ucn3 or L-Glu. The maximum inhibition in GSNA amplitude, in response to microinjections of L-Glu or Ucn3 into the mNTS, was expressed as a percent decrease from the control value of the GSNA amplitude. The mean values of the integrated nerve signals were compared using Student’s paired t-test. In all cases, the differences were considered significant at \( P < 0.05 \).

RESULTS

Baseline values for MAP and HR in urethane-anesthetized rats were 100.2 ± 1.9 mmHg and 395.8 ± 4.2 beats/min, respectively \((n = 46)\). The values for baseline MAP and HR in the decerebrate rats \((n = 6)\) were 102.5 ± 2.8 mmHg and 445.0 ± 27.0 beats/min, respectively. There were no significant differences \((P > 0.05)\) between the baseline values of MAP and HR in urethane-anesthetized and decerebrate rats.

Histology. The mNTS sites, where microinjections of L-Glu elicited depressor and bradycardic responses, were marked in 10 rats; a typical mNTS site marked with India ink (100 nl) is shown in Fig. 1A. Figure 1, B and C, represents composite diagrams of mNTS sites; the sites were located 0.5–0.6 mm rostral to the calamus scriptorius. The diminution of responses to the repeated administration of the same concentration of the agonist (tachyphylaxis) was also tested. The concentration of Ucn3 that elicited maximal cardiovascular responses (0.06 mM) was microinjected into mNTS at least four times at 30-min intervals \((n = 7)\). The decreases in MAP in response to four consecutive microinjections of Ucn3 (0.06 mM) were 18.5 ± 1.7, 18.5 ± 2.3, 18.5 ± 2.3, and 17.8 ± 2.8 mmHg, respectively, and the decreases in HR were 37.1 ± 11.0, 31.4 ± 6.7, 32.8 ± 2.6, and 29.2 ± 4.9 beats/min, respectively; a repeated-measure ANOVA showed that these values were not statistically different \((P > 0.05)\). Thus no tachyphylaxis of responses was observed with repeated microinjections of Ucn3 (0.06 mM) at 30-min intervals. Therefore, the interval between the microinjections of Ucn3 was at least 30 min in all experiments.

Site specificity of Ucn3-induced responses. The site specificity of Ucn3-induced responses was tested in another group of rats \((n = 4)\). First, the mNTS site was identified by microinjections of L-Glu, and Ucn3 (0.06 mM) was microinjected at the same site to elicit usual depressor and bradycardic responses. The same concentrations of L-Glu and Ucn3 were then microinjected into an adjacent site in the cuneate nucleus (0.5 mm rostral and 1.5 mm lateral to the calamus scriptorius and 0.5 mm ventral to the dorsal surface of the medulla). Neither L-Glu nor Ucn3 elicited any cardiovascular response at this site.

Effect of Ucn3 in decerebrate rats. The cardiovascular responses elicited by microinjections of Ucn3 (0.06 mM) into the
**Effect of CRFR antagonists on Ucn3-induced response.** The effects of a nonselective CRFR antagonist astressin on the cardiovascular responses to microinjections of Ucn3 into the mNTS were studied as follows. A site in the mNTS was identified on one side by microinjection of L-Glu (5 mM), and the initial depressor and bradycardic responses to Ucn3 (0.06 mM) were recorded. Within 30 min, astressin (1 mM) was microinjected at the same site. The depressor and bradycardic responses to Ucn3 microinjected 2 min after astressin were almost completely blocked. Sixty minutes after the blockade of CRFRs in the mNTS, the Ucn3-induced decreases in MAP and HR remained significantly reduced (Fig. 3, A and B). In the same group of rats, astressin did not alter the depressor and bradycardic responses to L-Glu (5 mM) microinjected into the same mNTS site within 5 min (Fig. 3, C and D). Microinjections of astressin alone did not elicit any appreciable effects on MAP and HR.

**Effect of CRF1-R antagonist on Ucn3-induced response.** The effect of a selective CRF1-R antagonist K41498 was also studied on the cardiovascular responses elicited by microinjections of Ucn3 (0.06 mM) into the mNTS. The mNTS site was identified on one side by a microinjection of L-Glu (5 mM). After the initial microinjection of Ucn3 (0.06 mM) into the mNTS, K41498 (5 mM) was microinjected at the same site within 30 min. The microinjection of Ucn3 (0.06 mM) was repeated at the same site within 2 and 60 min. Ucn3-induced decreases in MAP and HR were almost completely blocked by prior microinjections of K41498 and remained significantly

![Diagram](https://example.com/diagram.png)

**Fig. 2.** Cardiovascular effects of urocortin 3 (Ucn3). Concentration response (n = 12) for mean arterial pressure (MAP, A) and heart rate (HR, B). Microinjections (100 nl) of Ucn3 [0, 0.03, 0.06, 0.12, and 0.25 mM; 0 mM concentration refers to artificial cerebrospinal fluid (aCSF)] into the mNTS elicited the following decreases in MAP for 0.50 ± 1.0, 21.6 ± 2.6, 20.0 ± 2.8, and 12.7 ± 3.4 mmHg, respectively (Tukey-Kramer’s post hoc multiple comparison test). An ANOVA of HR values was found to be highly significant (F = 16.45, degrees of freedom = 4, 46, P < 0.0001). The depressor responses to 0.06 and 0.12 mM concentrations of Ucn3 were significantly greater than those of 0.03 mM concentration (**P < 0.01), and the depressor responses to 0.06, 0.12, and 0.25 mM concentrations of Ucn3 were significantly greater than those of 0 mM concentration (**P < 0.01) (Tukey-Kramer’s post hoc multiple comparison test). The decrease in HR elicited by 0.06 mM concentration was significantly greater than that elicited by 0.03 (**P < 0.01) (Tukey-Kramer’s post hoc multiple comparison test). C: the decreases in MAP induced by Ucn3 (0.06 mM) before and after bilateral vagotomy were 21.0 ± 1.8 and 18.0 ± 1.2 mmHg, respectively (P > 0.05) (n = 5). D: in the same group of rats, the decrease in HR (31.0 ± 6.7 beats/min) was completely blocked (****) after bilateral vagotomy. The baseline values for MAP before and after bilateral vagotomy were 29.0 ± 3.6 and 109.0 ± 2.9 mmHg, respectively (P > 0.05). The baseline values for HR before and after bilateral vagotomy were 40.8 ± 10.6 and 45.0 ± 8.3 beats/min, respectively (P < 0.05).

mNTS were compared between decerebrate (n = 6) and urethane-anesthetized (n = 30) rats. In the decerebrate and anesthetized rats, the decreases in MAP were 38.3 ± 5.1 and 25.5 ± 2.0 mmHg, respectively (P > 0.05), and decreases in HR were 56.6 ± 9.5 and 42.9 ± 4.0 beats/min, respectively (P > 0.05). These results indicated that urethane did not affect the cardiovascular responses to microinjections of Ucn3 into the mNTS.

**Effect of vagotomy on Ucn3-induced bradycardia.** A bilateral vagotomy did not significantly affect the decreases in MAP induced by a microinjection of Ucn3 (0.06 mM) into the mNTS (Fig. 2C). In the same group of rats, the Ucn3-induced decrease in HR was completely blocked after bilateral vagotomy (Fig. 2D). The baseline MAP was not significantly altered by bilateral vagotomy. However, the baseline HR after bilateral vagotomy was significantly greater compared with the value before bilateral vagotomy.

![Diagram](https://example.com/diagram.png)

**Fig. 3.** Effect of astressin on Ucn3-induced decreases in MAP and HR (n = 5). A and B: the initial decreases in MAP and HR by microinjection of Ucn3 (0.06 mM) into the mNTS were 23.0 ± 4.6 mmHg and 28.0 ± 3.7 beats/min, respectively. Two minutes after the microinjection of astressin (1 mM) into the mNTS, the Ucn3-induced decreases in MAP and HR were 2.0 ± 1.2 mmHg and 3.0 ± 3.0 beats/min, respectively (**P < 0.01). Sixty minutes later, the Ucn3-induced decreases in MAP and HR were still significantly smaller (4.0 ± 1.8 mmHg and 8.0 ± 3.7 beats/min, respectively, **P < 0.01). C and D: in the same group of rats, the decreases in MAP elicited by microinjections of L-glutamate (L-Glu; 5 mM) into the mNTS before and after the microinjection of astressin were 29.0 ± 4.0 and 30.0 ± 3.8 mmHg, respectively (P > 0.05). L-Glu-induced HR decreases before and after astressin were 45.0 ± 5.0 and 44.0 ± 4.0 beats/min, respectively (P > 0.05).
reduced 60 min after the blockade of CRF2Rs in the mNTS (Fig. 4, A and B). In the same group of rats, the depressor and bradycardic responses to microinjections of L-Glu (5 mM) were not significantly altered by K41498 (Fig. 4, C and D). The microinjection of K41498 alone did not exert any effect on MAP and HR.

A typical tracing showing the effect of K41498 on Ucn3-induced responses is shown in Fig. 5. The mNTS site was identified by a microinjection of L-Glu (5 mM, Fig. 5A). A microinjection of aCSF (100 nl), 2–5 min later, at the same site elicited no responses (Fig. 5B). About 2 min after the microinjection of aCSF, a microinjection of Ucn3 (0.06 mM) at the same site elicited decreases in MAP, pulsatile arterial pressure (PAP), and HR (Fig. 5C). Thirty minutes after the microinjection of Ucn3, K41498 (5 mM) was microinjected at the same site; the microinjection of K41498 alone did not elicit any effect on MAP, PAP, and HR (Fig. 5D). Within 2 min, Ucn3 (0.06 mM) was again microinjected at the same site; the responses to Ucn3 were blocked (Fig. 5E). K41498 did not alter the depressor and bradycardic responses to the microinjection of L-Glu (5 mM); the interval between the microinjections of K41498 and L-Glu was 5 min (Fig. 5F).

**Effect of Ucn3 on sympathetic nerve activity.** The effect of the microinjections of Ucn3 into mNTS on efferent sympathetic nerve activity (not shown). Within 2 min, Ucn3 (0.06 mM) was again microinjected at the same site; the responses to Ucn3 were blocked (Fig. 5E). K41498 did not alter the depressor and bradycardic responses to the microinjection of L-Glu (5 mM); the interval between the microinjections of K41498 and L-Glu was 5 min (Fig. 5F).

**DISCUSSION**

The main observation in this study was that microinjections of Ucn3 into the mNTS elicited depressor and bradycardic responses. This is the first report of the central cardiovascular effects of Ucn3 in the mNTS. Our results are in agreement with an earlier report of Yamazaki et al. (46) in which microinjections of Ucn1 and Ucn2 into the mNTS elicited depressor and
bradycardic responses. We extended these observations as follows: 1) a specific and potent agonist of CRF2Rs (Ucn3) was used; 2) using a specific antagonist, we demonstrated that the effects of Ucn3 were mediated via CRF2Rs in the mNTS; 3) we also established that Ucn3-induced depressor responses were mediated via the inhibition of sympathetic nerve activity, and bradycardic responses were mediated via the activation of vagal innervation to the heart; and 4) our experiments in unanesthetized midcollicular rats revealed that urethane anesthesia did not alter the responses to Ucn3 qualitatively or quantitatively and that the neural structures located rostral to the brainstem were not necessary for the observed Ucn3-induced cardiovascular effects in the mNTS.

Microinjections of Ucn3 into the mNTS showed a nonlinear bell-shaped concentration response. This type of concentration response has been reported for several enzymes, peptides, and hormones (4, 18). It has been explained by homotropic allosteric in which the agonist at higher concentrations binds to a modulator site, which is different from the primary binding site, and thereby affects the function of the receptor, resulting in an attenuated responses (1). Another explanation for the reduced responses at higher concentrations of Ucn3 could be that at higher concentrations, this agonist could activate inhibitory interneurons located in the mNTS. For example, the presence of GABAergic neurons and terminals (38) has been reported in the mNTS. The activation of these interneurons could release GABA in the mNTS, which is likely to increase BP and HR (38). Similarly, the presence of catecholaminergic neurons has been demonstrated in the mNTS, and the activation of these neurons may release norepinephrine in the NTS, which has been reported to increase BP (36). The pressor effects of GABA and norepinephrine in the NTS are expected to reduce the depressor and bradycardic responses to Ucn3 at higher concentrations. These possibilities for the reduced cardiovascular responses to higher concentrations of Ucn3 in the mNTS remain to be tested.

The possibility that the Ucn3-induced cardiovascular effects were due to its leakage from the microinjection site into the peripheral circulation was excluded because the doses of Ucn3 that elicited depressor and bradycardic responses when microinjected into the mNTS did not elicit responses when injected intravenously. The site specificity of Ucn3-induced cardiovascular responses was established by the lack of responses to Ucn3 microinjections into the areas located adjacent to mNTS, such as the cuneate nucleus. Consistent with our previous observations (3), the local distortion of brain tissue or any nonspecific effects were not responsible for the Ucn3-induced cardiovascular responses because the microinjections of aCSF into the mNTS did not elicit any responses.

It needs to be mentioned that the affinities of CRFRs are in the nanomolar range (21, 22), whereas the maximally effective concentration of Ucn3 used in this study was in the millimolar range. In view of the high concentration of Ucn3 used and the
high affinity of CRFRs, it is possible that Ucn3 may have reached the sites adjacent to the mNTS and contributed to the observed cardiovascular responses. Thus it is possible that Ucn3 may have diffused to the dorsal motor nucleus of vagus, which is located close to the mNTS. Although this nucleus has a minor role in the parasympathetic regulation of the HR and essentially no role in BP regulation, the participation of the dorsal motor nucleus of vagus in Ucn3-induced cardiovascular responses cannot be excluded with certainty.

Based on current knowledge regarding medullospinal cardiovascular regulatory areas (35), the mechanism of the cardiovascular responses elicited by microinjections of Ucn3 into the mNTS can be explained as follows. Ucn3 may stimulate mNTS neurons, which, in turn, may stimulate a population of GABAergic neurons located in the caudal ventrolateral medullary depressor area (CVLM). As a result, GABA is released in the rostral ventrolateral medullary pressor area (RVLM), causing a decrease in the activity of RVLM neurons. Consequently, the activity of the excitatory input from the RVLM neurons to the sympathetic preganglionic neurons located in the intermediolateral cell column of the thoracolumbar cord (IML) is decreased. Since the output of the sympathetic preganglionic neurons to the arterioles is decreased, the systemic BP decreases. Supporting this explanation is our observation that the sympathetic activity recorded from the central end of the GSN was decreased by microinjections of Ucn3 into the mNTS.

Although microinjections of Ucn3 into the mNTS may most likely elicit cardiovascular responses by activating the pathway involving mNTS, CVLM, RVLM, and IML, it is quite possible that other mNTS neurons following a direct pathway involving mNTS, RVLM, and IML may be activated to elicit opposite cardiovascular responses. In this context, it may be noted that microinjections of l-Glu into the mNTS normally elicit depressor and bradycardic responses in anesthetized and decerebrate animals by activating a pathway involving mNTS, CVLM, RVLM, and IML (9). However, when the CVLM is inhibited by microinjections of muscimol, l-Glu microinjections into the mNTS elicit pressor responses via the mNTS, RVLM, and IML pathway (41). If this pressor pathway is activated by Ucn3 microinjections into the mNTS, it may reduce the Ucn3-induced depressor and bradycardic responses elicited via the mNTS/CVLM/RVLM/IML pathway. This possibility remains to be tested.

The bradycardia elicited by a microinjection of Ucn3 into the mNTS was mediated via the activation of the vagal innervation to the heart. This conclusion was based on our observation that a bilateral vagotomy completely abolished Ucn3-induced bradycardic responses. The mechanism of Ucn3-induced bradycardic responses can be explained as follows, based on our current knowledge regarding the vagal control of the heart (25). The microinjections of Ucn3 into the mNTS may activate mNTS neurons, projecting to the nucleus ambiguus (nAmb). The parasympathetic preganglionic neurons located in the nAmb may be activated via the excitatory projection from the mNTS to the nAmb (29). The stimulation of parasympathetic preganglionic neurons in the nAmb may increase parasympathetic activity to the heart via the vagus nerves and elicit bradycardia.

Astrassin has been reported to be a highly potent antagonist at CRF1Rs as well as CRF2Rs (13, 21). On the other hand, K41498 has been reported to be a highly selective CRF2R antagonist (21). The bradycardic and depressor responses induced by microinjections of Ucn3 into the mNTS were almost completely blocked by a prior microinjection of astressin or K41498 at the same site. A Ucn3-induced decrease in GSNA was also blocked by K41498. These observations indicated that the cardiovascular responses induced by microinjections of Ucn3 into the mNTS were mediated via CRF2Rs. Astrassin and K41498 did not exert any deleterious effects at the site of injection because they did not alter responses to another unrelated agonist, l-Glu.

Microinjections of astrassin or K41498 alone into the mNTS did not elicit any cardiovascular responses. This observation suggested that in the mNTS, Ucn3 and CRF2Rs were not involved in the control of cardiovascular function under normal physiological situations. Although the presence of CRF2Rs in the mNTS has been reported (5, 8, 42), immunohistochemical studies on the rat brain have revealed the presence of Ucn1, but not Ucn3, fibers in the mNTS (2, 23). In addition, microinjections of Ucn1 into the mNTS have been reported to elicit depressor and bradycardic responses (46). Based on these reports, it appears that under normal physiological conditions, the endogenous ligand for CRF2Rs in the mNTS in the central regulation of cardiovascular function may be Ucn1 (46). However, Ucn3 may activate CRF2Rs in the mNTS under specific circumstances such as stress. For example, the presence of Ucn3 has been reported in human plasma, raising the possibility that this peptide may play a role as a circulating hormone (39). The source of Ucn3 in plasma is not known. The expression of Ucn3 has been reported in several peripheral tissues and organs, such as the heart, kidney, and different parts of the gastrointestinal tract (15, 22, 39). If the plasma levels of Ucn3 increase in certain situations (e.g., stress), there is a possibility that it may reach the mNTS via the area postrema and cause hypotension and bradycardia. Moreover, the fenestrated capillaries and perivascular spaces present in the NTS may permit a brisk influx of solutes into the neuropil of the mNTS (12). Hypotension caused by systemically infused Ucn2 and other CRF analogs (6, 33) may also be partially mediated via the activation of CRF2Rs in the mNTS because they cross the blood-brain barrier by a unique transport system (19). Acute stress is often associated with increases in BP and HR. Ucn3-induced hypotension and bradycardia may be beneficial under these situations.

In conclusion, microinjections of Ucn3 into the mNTS elicited depressor and bradycardic responses that were mediated via CRF2Rs. The depressor responses were mediated by a decrease in sympathetic nerve activity. The bradycardia was mediated via the vagus nerves. Ucn3 and CRF2Rs in the mNTS may be involved in cardiovascular regulation under yet-unidentified circumstances such as stress.

GRANTS

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REFERENCES


